Wildlife Health Laboratory
Standard Operating Procedures

The following Standard Operating Procedures (SOPs) are compliant with the CDC/NIH Biosafety Level 2 section of the Biosafety in Microbiological and Biomedical Laboratories (BMBL) guidelines. Laboratory personnel will read, understand and comply with relevant sections of these SOPs when working in the Wildlife Health Laboratories (WHL). Compliance with biosafe practices is the mandatory responsibility of each person using the WHL facilities.

Table of Contents: **Page**

Wildlife Health Laboratory
Standard Operating Procedures

A. Necropsy Laboratory

1. Title- General Guidelines

a. Purpose

To inform personnel using the WHL necropsy facility of general rules to be followed in the laboratory at all times. All laboratory activities are managed by the Wildlife Health Laboratory Supervisor. Most of the work conducted in the WHL necropsy lab is related to ongoing studies of chronic wasting disease (CWD) and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

b. Guidelines

i. Access to the necropsy lab is restricted to authorized personnel only.

ii. Equipment and supplies are not to be removed from the lab.

iii. Equipment and supplies are not for general DOW personnel use.

iv. No food or drink is permitted within the lab.

v. Laboratory SOP must be available to personnel on duty.

vi. MSDS for every chemical used in laboratory must be available to personnel on duty.

c. Wildlife Health Laboratory Supervisor

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A. Necropsy Laboratory

2. Title- Entry and Exit Procedures

a. Purpose

To inform personnel using the WHL necropsy facility of the proper entry and exit procedures. Most of the work conducted in the WHL necropsy lab is related to ongoing studies of chronic wasting disease (CWD) and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

b. Entry

i. The following protective clothing should be worn while performing necropsies on carcasses or extraction of tissue samples:

- Coveralls
- Plastic disposable apron
- Rubber boots or disposable plastic boots
- Eye protection
- Latex/nitrile gloves
- Cut-proof gloves (optional but highly recommended when processing carcasses)

ii. The following protective clothing should be worn when handling containerized tissue samples:

- Lab coat or coveralls
- Rubber boots or disposable plastic boots
- Eye protection
- Latex/nitrile gloves

c. Exit

i. Boots

a) Rubber boots are to be rinsed free of any organic material, and the entire surface to be scrubbed with 5% LpH while standing in 5% LpH boot bath before exiting lab.

b) Rubber boots are to be removed after exiting and placed in designated storage area.
c) Disposable plastic booties are to be removed at point of exit and disposed of in appropriate waste container within the lab.

ii. Clothing

a) Plastic apron is to be removed before exiting and disposed of in appropriate waste container within lab.
b) Coveralls are to be removed after exiting and hung in designated storage area if clean, or placed in container for washing if necessary.

iii. Hands

a) After removing apron and scrubbing boots, gloves are to be removed before exiting and disposed of in appropriate waste container within lab.
b) Hands and exposed areas of arms must be washed with antibacterial soap prior to exiting lab.

A. Necropsy Laboratory

3. Title- Decontamination Procedures

a. Purpose

To inform personnel using the WHL necropsy facility of the proper decontamination procedures for instruments and surfaces within the laboratory. Most of the work conducted in the WHL necropsy lab is related to ongoing studies of chronic wasting disease (CWD) and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

b. Instruments

i. All instruments used in the lab for necropsy or tissue dissection need to be decontaminated. This includes:

- Scalpel handles
- Scalpel blades
- Thumb forceps
- Tissue scissors
- Grapefruit knives
- Knives
- Cleavers
- Pliers
- Cutting boards
- Hand Saw

ii. Instrument Decontamination Procedure

1) Contaminated instruments are to be placed in a 5% LpH solution immediately after use to soak for >30 minutes. DO NOT place tooth knives in 5% LpH- it will destroy the handles.
2) Blades are removed from scalpel handles and placed in sharps container.
3) Instruments are then placed in an Inspector's Choice solution for >5 minutes, scrubbed to remove all tissue debris, and rinsed in lab sink. All water from the sink empties through mesh-covered drains.
4) Instruments are then rinsed thoroughly with water and placed into 5% LpH solution again for >30 minutes. DO NOT place tooth knives in 5% LpH- it will destroy the handles, and DO NOT leave instruments in LpH overnight- they will rust.
5) Instruments are then rinsed thoroughly with water, and placed in appropriate containers or storage locations.
6) DO NOT leave instruments in 5% LpH solution overnight, as it will rust the metal and destroy the plastic.

c. Surfaces

i. Necropsy laboratory surfaces used regularly include:

- Necropsy tables
- Floor (including cooler and freezer)
- Walls (including cooler and freezer)
- Shelves
- Carts and plastic bins
- Cement dropoff pad & walkway
- Trailers & truck beds

ii. Surface Decontamination Procedure

1) Floor should be misted with water prior to necropsy work and kept lightly wet during laboratory use.
2) Contaminated surfaces are coated entirely with 5% LpH solution using a pressurized hand sprayer or airchem machine and left to decontaminate for >30 minutes. Personnel are recommended to leave the laboratory during this time to avoid inhaling irritating fumes.
3) Entire surface is sprayed with Inspector's Choice solution using foamer attachment, and left for >5 minutes to loosen blood and/or tissue.
4) Surface is scrubbed with stiff brushes and rinsed clean with water.
5) Small squeegee is used to drain water off of necropsy tables.
6) Large squeegee is used to push all water on the floor into a mesh-covered trench drain.

d. Drains

i. Laboratory drains, each covered with wire mesh screen, include:

- 3 sink drains
- Floor trench drain on south wall of lab
- Floor trench drain in center of cement pad

ii. Drain Decontamination Procedure

1) Sink drains

a) Hose is used to spray inside of sink and push all debris into drain screen.
b) Screens containing debris are removed and replaced with clean, decontaminated screens on a weekly basis, or more often if needed.
c) Used screens are then cleaned out and all debris is deposited in appropriate waste container.
d) Used screens are placed in 5% LpH solution for >30 minutes, then rinsed.
e) Screens can be re-used after cleaning and decontamination.

2) Trench drains

a) Water and debris on floor/cement pad is squeegeed into trench drain.
b) Debris is collected from the wire mesh covering the drain and deposited in appropriate waste container.
c) Trench drain is then sprayed with 5% LpH and left to dry.

e. Spills

i. Biological spills include blood or tissue on the floor or dissecting tables, or other laboratory surfaces.

1) Biological Spill Decontamination Procedure

a) Affected area should be decontaminated as a surface according to section (A.3.c.) of the SOP.
b) Spill area can then be cleaned with the rest of the laboratory surfaces according to section (A.3.c.) of the SOP.

ii. Chemical spills include LpH, formalin, or other chemicals that occur on persons or clothing.

1) Chemical Spill Decontamination Procedure

a) Chemical spills should be handled as an emergency according to section (E.b.) of the SOP.
b) All chemical spills requiring medical attention must be recorded in the Emergency Log (Appendix III.d.), including the date, time, agents of exposure, and names of personnel exposed.

A. Necropsy Laboratory

4. Title- Maintenance

a. Purpose:

To inform personnel using the WHL necropsy facility of the proper daily, weekly, and monthly maintenance procedures. Most of the work conducted in the WHL necropsy lab is related to ongoing studies of chronic wasting disease (CWD) and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

b. Daily Maintenance

i. Daily cleaning and decontamination is the responsibility of personnel working in the facility.

ii. The necropsy laboratory is to be cleaned and decontaminated after use as described in section (A.3.) of the SOP.

iii. Temperature and water usage logs (Appendix III) are to be updated on a daily basis according to lab use.

iv. Chemicals are to be dated upon purchase, and recorded in chemical usage log (Appendix III) when opened.

v. All waste containers are to be emptied on a daily basis, and replaced with clean garbage bags. Waste is to be disposed of daily according to section (A.7.e.) of the SOP.

vi. The interior of the walk-in cooler and freezer will be cleaned and decontaminated on a weekly basis, or more often if necessary.

vii. Necessary repairs to the necropsy facility, including freezer, cooler, drain mesh, or shelving will be addressed immediately and repaired on an as-needed basis.

c. Weekly Maintenance

i. Supplies will be checked on a weekly basis by laboratory personnel to maintain an adequate supply of the following:

- Disposable gloves, aprons & boots
- Paper towels
- Garbage bags
- Ziploc Bags
- Whirlpacs (sample bags)
- Inspector's Choice Detergent
- LpH

ii. Screens covering trench drains and sink drains will be inspected and decontaminated on a weekly basis.

iii. Footbath and instrument bath containing 5% LpH solution will be changed at the start of each week.

iv. Cleaning and dusting of windows, sills, and shelves will be performed on a weekly basis.

d. Monthly Maintenance

i. Water usage log, found in Appendix III, is to be totaled and updated at the beginning of each month.

ii. Chemical usage logs, found in Appendix III, are to be updated and totaled on a monthly basis for the following chemicals:

- LpH
- Inspector's Choice Detergent
- Form-C (Formalin)
- Bleach (Sodium Hypochlorite)

A. Necropsy Laboratory

5. Handling of Formalin and Formalin-Fixed Tissues

a. Purpose

To inform personnel using the WHL necropsy facility of the proper procedures for handling formalin and formalin-fixed tissues. Most of the work conducted in the WHL necropsy lab is related to ongoing studies of chronic wasting disease (CWD) and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents, as well as limit exposure to potentially harmful chemicals including formalin.

b. General Formalin Handling Guidelines

i. Always use adequate ventilation when handling formalin to avoid direct inhalation:

- Turn ceiling vent on
- Open windows and overhead door
- Set up portable fan

ii. Coveralls, eye protection, Nitrile/latex gloves, and rubber boots must always be used when handling formalin.

iii. Always dilute formalin according to manufacturer's recommendations on label.

iv. Formalin spills must be rinsed immediately with large volumes of water.

c. Storage of Formalin

i. Concentrated formalin is to be stored in the manufacturer's 1-gallon container on a designated shelf within the lab.

ii. Diluted formalin is to be stored in a closed 5-gallon carboy on accessible shelf in lab.

iii. Used formalin is to be stored in a sealed carboy or 1-gallon container until disposed.

d. Fixing Tissues in Formalin

i. Cutting in of tissues must be performed by trained personnel.

ii. Fresh tissues are to be fixed in formalin in labeled cups for >24 hours.

iii. Fixed tissues are transferred with appropriate amount of formalin into labeled heavy-duty pouches, and immediately sealed with a heat-sealer.

iv. Instruments used to cut in tissues must be decontaminated according to section (3.b.) of the SOP.

e. Formalin material safety data sheet (MSDS)

- Appendix IV

A. Necropsy Laboratory

6. Title- Sample Extraction Procedures for Chronic Wasting Disease (CWD) Surveillance

a. Purpose:

To inform personnel using the WHL necropsy facility how to properly extract samples for chronic wasting disease (CWD) surveillance. Most of the work conducted in the WHL necropsy lab is related to ongoing studies of CWD and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to obtain the correct samples and limit personnel exposure to the CWD agent.

b. Cervid samples taken regularly in the necropsy laboratory for CWD surveillance include the following:

- Medial retropharyngeal lymph nodes
- Tonsils
- Brainstem, including obex
- Brain tissue (cerebrum and/or cerebellum)
- Front incisors
- Liver
- Colon (w/feces)
- Urine

c. General CWD Sampling Guidelines

i. Samples are to be removed by trained laboratory personnel only.

ii. Samples may be extracted in any order, except for the brain tissue, which must follow removal of the brainstem.

iii. All extracted samples from one animal must be placed in labeled sample bags before moving on to the next animal.

iv. Instruments that are to be re-used for more than one animal must be rinsed in 5% LpH.

v. After sampling instruments and surfaces are to be decontaminated according to section (3.) of the SOP.

d. Head Sample Extraction Procedures

i. Begin by placing the head on a layer of lab matting atop the necropsy table with the dorsal side down, and the neck facing the sampler.

ii. Palpate the ventral throat area and locate the pharynx.

iii. Using a scalpel, cut through the skin and proceed to sever the throat just rostral to the thyroid cartilage.

iv. Continue cutting, parallel to the ramus of the mandible, aiming towards the back of the ears. Stop cutting when the scalpel reaches the atlanto-occipital joint.

v. Samples

1) Medial retropharyngeal lymph nodes

a) Remove each (2) medial retropharyngeal lymph node from the roof of the laryngopharynx by grasping each with thumb forceps and cutting the surrounding tissue with a scalpel or scissors.

b) Take care not to mistake salivary tissue, muscle tissue, or pharyngeal pouch tissue for lymph nodes.

c) Place each lymph node in designated sample bag.

2) Tonsils

a) Remove each (2) tonsil from the soft palate by grasping the tonsillar crypt with thumb forceps and cutting around the bulge of the tonsil with a scalpel.

b) Remove as much surrounding tissue as possible and place tonsils together in single designated sample bag.

3) **Brainstem, including obex**

a) Using a scalpel, separate the atlanto-occipital joint by cutting ligaments on either side of the joint and severing the spinal cord in the center of the joint.

b) Use scissors or a scalpel to cut the cranial nerves and dural attachments around the brainstem.

c) Reach into the foramen magnum with a scalpel or grapefruit knife and sever the brainstem above the region of the obex.

d) Place the brainstem, including the obex, into designated sample bag or cup.

4) **Brain tissue**

a) Using a scalpel or grapefruit knife, reach into foramen magum after brainstem has been removed and scoop out a spoon-sized piece of brain tissue, either cerebrum or cerebellum.

b) Place brain tissue in designated sample bag or cup, to be placed in cooler, freezer or formalin as required.

5) **Front incisors**

a) Using a large knife, slice the gum tissue between the front two incisors and on either side of the incisors to remove the teeth.

b) Place teeth in small, labeled envelope and leave envelope open until teeth have dried out to prevent them from molding or rotting.

e. Carcass Sample Extraction Procedures

i. Liver

1) Using a scalpel, cut along most caudal region of ribcage on the right side of the carcass, making a 4-8 inch incision. Try to avoid puncturing the rumen.
2) Reach into abdominal cavity, dorsally along the diaphragm, feeling for the smooth, firm surface and lobes of the liver.
3) Pull off a handful-sized intact chunk of liver and place in designated sample bag.

ii. Colon

1) Using a scalpel, cut through inner thigh muscles of one hind leg to access pelvic region.
2) Cut through wall of abdominal cavity along the pubis and move intestines out of the way.
3) Reach into cavity and grasp colon, which is lying along dorsal surface of cavity.
4) Remove a 12 inch section of colon, as caudal as possible.
5) Leave feces in colon and place in designated sample bag.

iii. Urine

1) Enter abdominal cavity as described above for colon extraction.
2) Find the bladder, which will be tucked caudally just dorsal to the pelvic symphysis.
3) A full bladder will have palpable fluid in it; an empty bladder will be quite small.
4) If bladder is full or partially full, collect as much urine as possible using a 1 ½ inch 18 gauge hypodermic needle and 20 cc syringe.
5) Place urine into a 50 ml tube or 200 ml sample cup and label.

A. Necropsy Laboratory

7. Title- Waste Disposal

a. Purpose:

To inform personnel using the WHL necropsy facility how to properly dispose of heads, carcasses, tissues, sharps and other laboratory waste. Most of the work conducted in the WHL necropsy lab is related to ongoing studies of CWD and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

b. Head and Carcass Disposal

i. CWD Suspect Disposal

1) Suspect heads and carcasses include the following:

- Hunter-harvested animals
- Roadkilled animals
- Culled animals
- FWRF research animals
- Free-ranging suspects

2) Heads and carcasses from the endemic area (GMU's: 7, 8, 9, 19, 191, 20, 29, 38, 87, 88, 89, 90, 91, 92, 93, 94, 95, 951) are to be placed in a designated trailer after sampling, to be disposed of by one of the following two methods:

a) Colorado State University Digester

- The digester completely breaks down and inactivates all organic material into liquid using highly alkaline chemicals (including NaOH) under high pressure.

- The liquid is evaporated, leaving a low-volume, non-infectious powder that can be disposed of.

b) Incinerator (if available)

- The incinerator uses a 2-chamber system.
- Heads and carcasses are combusted at temperatures ranging from 1500-1600 °C in the primary chamber, producing a gas.
- The gas undergoes additional combustion at 1900 °C in the secondary chamber to breakdown any remaining particles.
- The remaining ash left after incineration is bagged and disposed of in a landfill

3) Heads and carcasses from the non-endemic area (all other GMU's) are to be placed in the designated trailer after sampling, and taken to the landfill. They may, if room allows, also be taken to the incinerator or digester.

ii. Non-CWD Suspect Disposal

1) Non –CWD suspect heads and carcasses are treated as those from the non-endemic area; they are to be placed in the designated trailer after sampling, and taken to the landfill. They may, if room allows, also be taken to the incinerator or digester.

iii. Zoonotic Disease Suspects

1) Other diseases of potentially zoonotic nature include:

- Rabies
- Plague
- Tularemia
- West Nile Virus

2) Carcasses or body parts from potentially zoonotic cases must be disposed of via the incinerator or digester.

iv. Other Terrestrial/Aquatic Species Disposal

1) The disposal of other species is to be handled on a case-by-case basis, as determined appropriate by the Laboratory Supervisor.

c. **Tissue Disposal**

i. **CWD Tissues**

1) All tissues associated with CWD suspect heads and carcasses are to be handled at a minimum.

2) Any laboratory surfaces contacted by potential CWD suspect tissues must be decontaminated according to section (A. 3.c.) of the SOP.

3) Small bits of tissue, >10 grams, may be collected and placed in designated waste container, and taken to the landfill.

4) Large bits of tissue and body parts must be treated as carcasses and handled according to section (A.7.b.) of the SOP, based on endemic or non-endemic status of the animal they came from.

iii. **Zoonotic Disease Tissues**

1) Tissues from potentially zoonotic cases must be disposed of via the incinerator or digester.

iv. **Non-Infectious Tissues**

1) Tissues not associated with infectious or zoonotic diseases may be disposed of in the landfill.

2) Unusual tissues must be reported to the Laboratory Supervisor, and will be handled on a case-by-case basis.

3) Tissues in question will be treated as suspect and disposed of by incineration or digestion.

d. Sharps Disposal

i. Sharps include:

- Removable scalpel blades
- Disposable scalpels
- Hypodermic needles
- Broken glass

ii. Used scalpel blades are to be removed from blade handle using pliers, and placed in sharps container.

iii. Used needles are to be placed directly into sharps container.

iv. Full sharps containers are to be permanently sealed, autoclaved at 121 °C for one hour, then incinerated at the Wyoming State Veterinary Laboratory.

e. General Waste Disposal

i. Regular laboratory waste includes:

- Used gloves and aprons
- Plastic bags
- Paper towels
- Cardboard boxes
- Lab matting
- Drain collection
- Plastic booties

ii. General waste is to be collected in waste containers lined with large garbage bags, and placed in the facility dumpster to be disposed of in the landfill.

B. Molecular Biology Laboratory

1. Title- General Guidelines

a. Purpose:

To inform personnel using the WHL molecular biology laboratory of general rules, including use of the biosafety cabinet, to be followed at all times. All laboratory activities are managed by the Wildlife Health Laboratory Supervisor. Most of the work conducted in the WHL molecular biology lab is related to ongoing studies of chronic wasting disease (CWD) and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

b. Guidelines

i. Access to the laboratory is restricted to authorized personnel only.

ii. No food or drink is permitted within the laboratory.

iii. Molecular laboratory instruments MAY NOT be used in the DNA/PCR laboratory.

vii. Laboratory SOP must be available within laboratory to personnel on duty.

iv. A BL2 biohazard sign must be posted on entry door to lab, indicating the agents in use, laboratory supervisor, and contact numbers.

v. MSDS for every chemical used in laboratory must be available to personnel on duty. MSDS are located in Appendix VI of the SOP, as well as in the molecular biology laboratory.

vi. Always remove protective clothing and wash hands before exiting lab.

vii. Shut down biosafety cabinet and turn off lights before exiting lab at the end of the day.

c. Biosafety Cabinet (BSC)

i. All manipulation of tissues that may potentially cause splattering or aerosolization must be performed in the BSC.

ii. The blower and light are to be turned on prior to beginning any work in the BSC.

iii. The working surface of the BSC is to be covered with absorbent, plastic-lined lab matting, with plastic side down. The lab matting must be changed at the end of the day, between tissue types, or more frequently if spilled on or contaminated.

iv. The BSC must be certified on an annual basis. BSC certification is located on the hood itself.

d. Wildlife Health Laboratory Supervisor

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B. Molecular Biology Laboratory

2. Title- Entry and Exit Procedures

a. Purpose:

To inform personnel using the WHL molecular biology laboratory of the proper entry and exit procedures. Most of the work conducted in the WHL molecular biology lab is related to ongoing studies of chronic wasting disease (CWD) and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

b. Entry

i. The following protective clothing should be worn at all times in the molecular biology laboratory:

- Lab coat
- Eye protection
- Latex/nitrile gloves
- Close-toed shoes

ii. A current logbook must be posted near entry to the lab containing a record of all spills or personnel exposure requiring medical attention. A permanent record is necessary due to the long incubation time of TSE's.

c. Exit

i. Lab coat, eye protection, and gloves are not to be worn outside the laboratory, and must be removed before exiting.

ii. Hands must be washed with soap before exiting laboratory.

B. Molecular Biology Laboratory

3. Title- Decontamination Procedures

a. Purpose:

To inform personnel using the WHL molecular biology laboratory of the decontamination procedures for instruments, equipment, surfaces, fluids and spills. Most of the work conducted in the WHL molecular biology lab is related to ongoing studies of chronic wasting disease (CWD) and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

b. Instruments

i. All instruments used for tissue preparation or assays must be decontaminated. This includes:

- Thumb forceps
- Scalpel handles
- Cutting boards
- Blender

ii. Instrument Decontamination Procedure

1) Contaminated instruments are to be placed in a 5% LpH bath immediately after use to soak for >30 minutes.

2) Blades are to be placed in sharps container after decontamination.

3) Instruments are to be rinsed thoroughly with water and placed in drying rack.

4) Instruments are to be put away in lab when dry.

c. Equipment

i. All equipment used for tissue preparation or assays must be decontaminated. This includes:

- Pipettes
- Scales
- Incubators
- Electrophoresis Boxes
- Blotters
- Vortexes
- Centrifuges

ii. Equipment Decontamination Procedure

1) All items that may have become contaminated must be wiped down after use with a 5% LpH solution and left to dry. Use squirt bottles for dispensing LpH to avoid aerosolization.

2) After each use, electrophoresis boxes should be rinsed with 5% LpH solution and left for >30 minutes, then rinsed with water.

d. Surfaces

i. Molecular biology laboratory surfaces include:

- Biosafety cabinet (BSC)
- Countertops
- Refrigerator
- Floor

ii. The BSC, countertops, and refrigerator surfaces must be decontaminated on a daily basis when the lab is in use.

iii. The floor must be decontaminated when spills or other contamination occurs.

iv. Surface Decontamination Procedure

1) The biosafety cabinet is to be wiped on the inside with a 5% LpH solution and left to dry. Surfaces may be wiped with water after 30 minutes if a film remains. Glass is to be wiped with a 70% isopropyl alcohol solution.

2) Countertops are to be wiped with a 50% bleach (sodium hypochlorite) solution and left to dry. Do not use LpH on countertops.

3) Refrigerator doors are to be wiped with a 5% LpH solution.

4) Floor is to be decontaminated with 5% LpH only in occurance of a spill. See section (B.3.f.) of the Molecular Biology lab SOP for cleanup of spills.

e. Fluids

i. All fluids used in CWD tissue processing or assays must be decontaminated. This includes:

- Used buffers
- Pipet contaminated liquids

ii. Fluid Decontamination Procedure

1) Small volumes (<50 ml) of contaminated liquids must be treated by one of the following procedures:

- Place fluid in a 50 ml disposable plastic tube (w/cap) containing ~10 ml concentrated LpH for >30 minutes, then dispose of in biohazard waste container.

- Place fluid in 50 ml disposable plastic tube (w/cap) containing ~25 ml concentrated bleach (sodium hypochlorite) for 1 hour, then poured fluid down the drain with running water and dispose of tube in biohazard waste container.

2) Large volumes (>50 ml) of contaminated liquids must be treated by one of the following procedures:

- Add concentrated LpH to fluid to a minimum of 5% of total fluid, and let sit for >30 minutes in covered container. Fluid may then be washed down the drain with running water.

- Add concentrated bleach (sodium hypochlorite) equal in volume to contaminated liquid, achieving a 50% bleach solution, let sit for >1 hour, then wash fluid down the drain with running water.

f. Spills

iii. Spills Decontamination Procedure

1) Affected area should be wetted immediately using 5% LpH from a squirt bottle; enough LpH should be used to adequately cover the entire spill.

2) Spill area should then be covered with paper towels and left for >30 minutes.

c) If spill occurs in the biosafety cabinet, the fan should run during the 30-minute period.

d) Contaminated materials are to be placed directly into biohazard waste container.

e) After spill has been removed, the entire area or in such cases, the entire biosafety cabinet, must be wiped down with 5% LpH and allowed to dry.

iv. Spills that occur on persons or clothing should be handled as an emergency according to section (E.) of the SOP.

v. All spills requiring medical attention must be recorded in laboratory logbook, including the date, time, agents of exposure, and names of personnel exposed.

B. Molecular Biology Laboratory

4. Title- Maintenance

a. Purpose:

To inform personnel using the WHL molecular biology laboratory of the proper daily, weekly, and annual maintenance procedures. Most of the work conducted in the WHL molecular biology lab is related to ongoing studies of chronic wasting disease (CWD) and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

b. Daily Maintenance

i. Daily cleaning and decontamination is the responsibility of personnel working in the laboratory.

ii. The laboratory is to be cleaned and decontaminated after use as described in section (B.3.) of the SOP.

iii. Biohazard bags are to be checked daily and if full, removed and autoclaved at 121 °C for 1 hour. Autoclaved bags are to be saved for incineration. Containers must be replaced with new biohazard bags.

iv. Necessary repairs to the refrigerator, freezer, biosafety cabinet, or other laboratory equipment will be addressed immediately and repaired on an as-needed basis.

v. Chemical usage must be noted in log every time a new liter of bleach is opened, or liter of LpH solution is made, according to section (H.) of the SOP.

c. Weekly Maintenance

i. Supplies will be checked on a weekly basis by laboratory personnel to maintain an adequate supply of the following:

- Latex/nitrile gloves
- Paper towels
- Biohazard bags
- Bleach (sodium hypochlorite)
- LpH
- Deionized H_20

ii. Items needed for specific laboratory procedures may be added to the supply list.

iii. Laboratory floor should be swept and mopped with anitbacterial cleaner on a weekly basis.

d. Annual Maintenance

i. Biosafety cabinet must be inspected and certified annually to maintain proper certification.

B. Molecular Biology Laboratory

5. Title- Handling of Tissues

a. Purpose:

To inform personnel using the WHL molecular biology laboratory of the proper procedures for handling and preparing tissues. Most of the work conducted in the WHL molecular biology lab is related to ongoing studies of chronic wasting disease (CWD) and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

b. CWD Tissues

i. Both fresh and frozen tissues must be contained in a sample cup or bag until processed.

ii. Frozen tissues should be kept in freezer until processing, and returned to freezer immediately after processing to maintain integrity of tissue.

iii. Tissues must be removed from numbered sample bag/cup one at a time, and returned to sample bag/cup

iv. Cutting and weighing of CWD tissues may be done on an open countertop.

v. Any procedure that could potentially aerosolize or splatter tissue, such as grinding or blending, must be performed in the biosafety cabinet.

c. Non-CWD Tissues

i. Other tissues should be handled with the same precaution as CWD tissues, unless otherwise directed by the laboratory supervisor.

B. Molecular Biology Laboratory

6. Title- Assay Procedures

a. Purpose:

To inform personnel using the WHL molecular biology laboratory of the standard assays performed in the lab, and the proper protocols. Most of the work conducted in the WHL molecular biology lab is related to ongoing studies of chronic wasting disease (CWD) and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

- **See Appendix III for specific protocols.**

a. **Gel Electrophoresis-** This procedure is used in several different protocols for the purpose of separating proteins by molecular weight using electrical current. The separated proteins can then be stained, or transferred onto a membrane and chemically illuminated.

b. **Western Blot-** This procedure is used to transfer proteins, separated by gel electrophoresis, onto a membrane for chemical illumination.

c. **Dot Blot ELISA-** This test is an enzyme-linked immunosorbent assay. (ELISA)

d. **Lateral Flow Test-** This rapid-testing procedure is undergoing trials to determine its potential use as a live-animal field test.

e. **Dialysis-** This procedure is used as an initial step to concentrate fluids, such as urine, to achieve higher sensitivity in assays.

B. Molecular Biology Laboratory

7. Title- Laboratory Waste Disposal

a. Purpose:

To inform personnel using the WHL molecular biology laboratory of how to properly dispose of tissues, equipment, sharps, and other lab waste. Most of the work conducted in the WHL molecular biology lab is related to ongoing studies of chronic wasting disease (CWD) and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

b. Tissues

i. Any excess, leftover, or processed tissues in the laboratory must be treated with 5% LpH solution for >30 minutes, and placed in a biohazard bag.

ii. All biohazard bags are autoclaved at 121 °C for 1 hour and then taken to Wyoming State Veterinary Laboratory to be incinerated.

c. Fluids

i. Treated fluids may be autoclaved within respective containers, or washed down the drain with copious amounts of water.

ii. Used decontamination fluids, including 5% LpH and 50% bleach (Sodium Hypochlorite), may be washed down the drain with copius amounts of water.

d. Equipment

i. Equipment that may be disposed of after use in the laboratory includes:

- Pipette tips
- Tubes and lids
- Disposable pipettes
- Biopsy punches
- Assay plates

ii. Disposable equipment must be placed in a 5% LpH bath for >30 minutes for decontamination.

iii. Equipment can then be placed in biohazard bag.

iv. All biohazard bags are autoclaved at 121 °C for 1 hour and then taken to Wyoming State Veterinary Laboratory to be incinerated.

e. Sharps

i. Sharps used in the laboratory include:

- Disposable scalpels
- Scalpel or razor blades
- Hypodermic needles

ii. Sharps are to be placed in sharps container immediately after use.

v. When full, sharps container is to be sealed properly and autoclaved at 121 °C for 1 hour.

vi. Sharps containers are then taken to the Wyoming State Veterinary Laboratory to be incinerated.

f. Other lab waste

i. All other laboratory waste, including gloves, paper towels, and lab matting, must be placed in a biohazard bag.

ii. When a biohazard bag is full, it should be wrapped with autoclave tape, then autoclaved at 121 °C for 1 hour.

iii. All biohazard bags are then taken to the Wyoming State Veterinary Laboratory to be incinerated.

C. DNA/PCR Laboratory

1. Title- General Guidelines

a. Purpose:

To inform personnel using the WHL DNA/PCR laboratory of general rules to be followed at all times. All laboratory activities are managed by the Wildlife Health Laboratory Supervisor. Most of the work conducted in the WHL DNA/PCR lab is related to ongoing field studies of wildlife populations and diseases. These guidelines are necessary to maintain proper practices in order to limit possible exposure of personnel to diseases.

b. Guidelines

i. Access to the DNA/PCR lab is restricted to authorized personnel only.

ii. No food or drink is permitted within the lab.

iii. Always wash hands with soap immediately upon exiting the DNA/PCR lab.

iv. All CWD tissues except for blood must be taken into the molecular biology lab for processing.

v. PCR/DNA lab instruments MAY NOT be used in the molecular biology lab.

vi. Laboratory SOP must be available to personnel on duty.

vii. MSDS for every chemical used in laboratory must be available on lab shelf to personnel on duty.

c. Wildlife Health Laboratory Supervisor

- Dr. Laurie A. Baeten, DVM
  Office: 970-416-1516
  Pager: 970-490-5028
  Cell: 970-443-2878

C. DNA/PCR Laboratory

2. Title- Entry and Exit Procedures

a. Purpose:

To inform personnel using the WHL DNA/PCR laboratory of the proper entry and exit procedures. Most of the work conducted in the WHL DNA/PCR lab is related to ongoing field studies of wildlife populations and diseases. These guidelines are necessary to maintain proper practices in order to limit possible exposure of personnel to diseases.

b. Entry

i. Gloves should be worn at all times when working in the DNA/PCR lab.

ii. A lab coat and eye protection are recommended when working in the DNA/PCR lab.

c. Exit

i. Lab coat, eye protection, and gloves are not to be worn outside the laboratory, and must be removed before exiting.

ii. Hands must be washed with soap before exiting laboratory.

C. DNA/PCR Laboratory

3. Title- Decontamination Procedures

a. Purpose:

To inform personnel using the WHL DNA laboratory of the decontamination procedures for instruments, equipment, surfaces, fluids and spills. Most of the work conducted in the WHL DNA lab is related to ongoing field studies of wildlife populations and diseases. These guidelines are necessary to maintain proper practices in order to limit possible exposure of personnel to diseases.

b. Decontamination Solvents

i. 50% bleach (sodium hypochlorite) must be used to decontaminate any instruments, equipment, surfaces, or fluids that come into contact with CWD tissue or bodily fluids.

ii. Eliminase must be used to decontaminate any instruments, equipment, surfaces, or fluids that come into contact with DNA or RNA.

iii. A viricide must be used to decontaminate any instruments, equipment, surfaces, or fluids that come into contact with potentially virally infected tissues or bodily fluids.

c. Instruments & Equipment

i. Instruments and equipment used in the DNA laboratory include:

- Pipettes
- Disposable pipettes
- Scales
- Incubators
- Vortexes
- Centrifuges
- Thermal cycler
- UV Camera
- Agarose gel electrophoresis machine
- Light microscope

ii. All instruments and equipment used in the DNA lab must be decontaminated or disposed of according to use.

iii. Decontamination Procedures

1) Disposable instruments used in the DNA lab are to be placed in a biohazard waste container to be autoclaved.

2) Non-disposable instruments are to be decontaminated using appropriate solvent according to section (C.3.b.) of the SOP.

3) Sharps are to be disposed of immediately in sharps container.

d. Surfaces

i. DNA laboratory surfaces include:

- Biosafety cabinet
- Countertops
- Refrigerator & freezer
- Floor

ii. Surface Decontamination Procedure

1) Biosafety cabinet is to be wiped on the inside with appropriate solvent according to section (C.3.b.) of the SOP. Glass is to be wiped with a 70% isopropyl alcohol solution.

2) Countertops are to be wiped with appropriate solvent according to section (C.3.b.) of the SOP.

3) Refrigerator freezer doors are to be wiped with appropriate solvent according to section (C.3.b.) of the SOP.

4) Floor is to be decontaminated with 50% bleach only in occurance of a spill. See section (C.3.f.) of the DNA laboratory SOP for cleanup of spills.

e. Fluids

i. Fluids and their containers must be properly disposed of. This includes:

- Buffers
- Blood
- Serum tubes
- EDTA tubes
- DNA vials
- Syringes

ii. Fluid Procedures

1) All containers holding biological fluids (blood, urine, etc) must be placed in a biohazard waste container to be autoclaved.

2) CWD-contaminated fluids must be treated accordint to section (B.3.e.) of the SOP.

3) Non-biological fluids may be poured down the sink with running water. Note: bleach should NOT be poured down the sink unless heavily diluted with running water, as it can affect the septic system.

f. Spills

i. Spill Cleanup Procedure

1) Personnel must always wear gloves when cleaning up a spill.

2) Affected area should be covered immediately with paper towels, absorbed, and dried. Spill area should then be wiped with 50% bleach, and left to dry.

3) If spill occurs in the biosafety cabinet, the fan should run during cleanup.

4) Materials used during cleanup are to be placed directly into biohazard waste container.

ii. Spills that occur on persons or clothing should be handled as an emergency according to section (E.) of the SOP.

iii. All spills involving potential exposure must be recorded in laboratory logbook, including the date, time, agents of exposure, and names of personnel exposed.

C. DNA/PCR Laboratory

4. Title- Laboratory Maintenance

a. Purpose:

To inform personnel using the WHL DNA laboratory of the proper daily, weekly, and annual maintenance procedures. Most of the work conducted in the WHL DNA lab is related to ongoing field studies of wildlife populations and diseases. These guidelines are necessary to maintain proper practices in order to limit possible exposure of personnel to diseases.

b. Daily Maintenance

i. Daily cleaning is the responsibility of personnel working in the laboratory.

ii. The laboratory is to be cleaned after use as described in section (C.3.) of the SOP.

iii. The (-70 °C) temperature log is to be updated on a daily basis according to lab use as described in section (H.) of the SOP.

iv. Biohazard waste containers are to be checked daily and if full, removed and autoclaved at 121 °C for 1 hour. Autoclaved bags are to be saved for incineration. Containers must be replaced with new biohazard bags.

v. Necessary repairs to the refrigerator, freezer, biosafety cabinet, or other laboratory equipment will be addressed immediately and repaired on an as-needed basis.

c. Weekly Maintenance

i. Supplies will be checked on a weekly basis by laboratory personnel to maintain an adequate supply of the following:

- Latex/nitrile gloves
- Paper towels
- Biohazard bags
- Bleach (sodium hypochlorite)
- Eliminase
- Viricide

ii. Items needed for specific laboratory procedures may be added to the supply list.

iii. Laboratory floor should be swept out and mopped with anitbacterial cleaner on a weekly basis.

d. Annual Maintenance

ii. Biosafety cabinet must be inspected and approved annually to maintain proper certification.

C. DNA/PCR Laboratory

5. Title- Handling and Storage of Biological Samples

a. Purpose:

To inform personnel using the WHL DNA laboratory of the proper procedures for handling and storage of tissues. Most of the work conducted in the WHL DNA lab is related to ongoing field studies of wildlife populations and diseases. These guidelines are necessary to maintain proper practices in order to limit possible exposure of personnel to diseases.

b. Handling of Biological Samples

i. All biological samples must be handled wearing latex/nitrile gloves.

ii. Surfaces should be covered with lab matting before handling samples.

iii. Tubes containing blood and/or blood products are to remain capped during transport and centrifugation.

iv. Used tubes that are either empty or still contain blood products are to be placed in biohazard waste container.

c. Storage of Biological Samples

i. All biological samples are to be properly labeled with contents, sample # and date before storage in the refrigerator or freezer.

ii. CWD samples may be stored in the (-70 °C) freezer or refrigerator located in the DNA laboratory and should be double packaged. CWD samples MUST NOT be removed from their packaging within the DNA laboratory; they must be taken into the molecular biology laboratory.

C. DNA/PCR Laboratory

6. Title- Laboratory Waste Disposal

a. Purpose:

To inform personnel using the WHL DNA laboratory of how to properly dispose of tissues, equipment, sharps, and other lab waste. Most of the work conducted in the WHL DNA lab is related to ongoing field studies of wildlife populations and diseases. These guidelines are necessary to maintain proper practices in order to limit possible exposure of personnel to diseases.

b. Biological Samples

i. Any excess, leftover, or processed biological samples and their containers are to be placed in a biohazard waste container.

ii. All biohazard bags are autoclaved at 121 °C for 1 hour and then taken to Wyoming State Veterinary Laboratory to be incinerated.

g. Equipment

i. Equipment that may be disposed of after use in the laboratory includes:

- Pipette tips
- Tubes, vials and lids
- Disposable pipettes
- Assay plates
- Syringes

ii. Disposable equipment must be placed directly into a biohazard waste container.

iii. All biohazard bags are autoclaved at 121 °C for 1 hour and then taken to Wyoming State Veterinary Laboratory to be incinerated.

h. Sharps

i. Sharps used in the laboratory include:

- Scalpel or razor blades
- Hypodermic needles

ii. Sharps are to be placed in sharps container immediately after use.

iii. When full, sharps container is to be sealed properly and autoclaved at 121 °C for 1 hour.

iv. Sharps containers are then taken to the Wyoming State Veterinary Laboratory to be incinerated.

i. Other lab waste

i. Laboratory waste that may have come into contact with biological samples, such as soiled gloves or lab matting, must be placed in a biohazard waste container. All biohazard bags are autoclaved at 121 °C for 1 hour and then taken to Wyoming State Veterinary Laboratory to be incinerated.

ii. Other laboratory waste, such as packaging materials, may be placed into a regular waste container. Standard, non-biohazard waste from the DNA laboratory and offices is taken to the landfill.

E. Title- Emergencies and Exposures of Personnel to Hazardous Material

a. Purpose:

To inform personnel of the WHL facility of laboratory emergency procedures for handling hazardous exposure, medical emergencies, and fire. Most of the work conducted in the WHL facility is related to ongoing studies of CWD and other wildlife diseases that are potentially zoonotic in nature. These guidelines are necessary to limit personnel exposure and transmission of zoonotic agents.

b. Exposures of Personnel to Hazardous Material

i. General

Exposures of personnel to hazardous material include exposure to BL2 chronic wasting disease TSE agent or other zoonotic disease agents, as well as exposure to potentially dangerous chemicals used within the lab. Research has shown that minor exposure to TSE agents are unlikely to eventually cause disease, and there are no known cases of humans becoming infected with CWD.

ii. Emergency Kit

Each laboratory in the WHL facility must contain the following emergency supplies:

- 500 ml squirt bottle of 5% LpH
- 500 ml squirt bottle of water
- eyewash bottle containing saline solution

iii. Spills

1) Spills must be contained, cleaned up and decontaminated immediately to avoid unnecessary exposure to laboratory personnel.

- If spill occurs on personnel, immediately remove contaminated clothing according to section (E.b.iii.5) and follow the SOP below.
- For spills that occur in the necropsy laboratory, please refer to section (A.3.e.) of the SOP.
- For spills that occur in the microbiology laboratory, please refer to section (B.3.f.) of the SOP.

iv. Exposure to Zoonotic Agent:

1) Contact with Unbroken Skin:

a) Gently wash area exposed to agent with 5% LpH over sink.
b) Wash with soap and copious amounts of water, rinse and pat dry. IMPORTANT- avoid scrubbing vigorously to prevent skin abrasions!

2) Cuts, Scrapes or Punctures:

a) For wounds that penetrate the skin with CWD contaminated instruments, such as scalpels or needles, immediately remove and dispose of gloves or other protective clothing at site of wound.
b) Gently encourage bleeding for a few minutes.
c) Wash several times with soap and water, then pat dry.
d) Cover wound with appropriate bandage.

3) Exposure to Eyes, Ears, Nose or Mouth:

   a) DO NOT use 5% LpH for eyes, ears, nose or mouth.
   b) Use saline solution or water to repeatedly wash contaminated area.
   c) NOTE: facial area should be protected at all times by eye protection and, in the molecular biology laboratory, use of the hood.

4) Accidental Ingestion

   a) Immediately induce vomiting.
   b) Consult immediately with laboratory supervisor or designated emergency contacts.
   c) Contain and decontaminate vomit with 5% LpH solution according to section (B.3.f.) of the SOP.

5) Contaminated Garments

   a) If protective clothing becomes contaminated during a procedure or spill, immediately remove garment and squirt contaminated area with 5% LpH.
   b) Place garment in biohazard waste container, don new garments as necessary, and continue spill decontamination procedure.

c. Other Medical Emergencies

i. In the case of a medical emergency, dial 911 immediately and stay in phone contact as long as needed.

ii. If the individual can be moved, remove them from the BL2 facility.

iii. If the individual is unconscious, immediately begin first aid.

iv. If the individual is not breathing, immediately begin CPR and continue until emergency medical personnel arrive and take over.

v. In critical situations, emergency medical personnel can enter BL2 laboratories without protective clothing or procedures; however, if the situation allows they should be instructed to don protective clothing.

d. Fire

i. Call 911 immediately from cell phone or from office at the pens.

ii. In the case of a fire in the WHL facility, attempt to extinguish flames using fire extinguisher (under sink in each lab) and aiming at the base of the flames.

iii. If fire persists or gets larger, all personnel must immediately exit both buildings and stand back >100 yards.

iv. Maintain distance from buildings until emergency personnel arrive and assess situation.

e. Emergency Contacts:

- Dr. Laurie Baeten, DVM, Laboratory Supervisor

  Office: 970-416-1516
  Pager: 970-490-5028
  Cell: 970-443-2878
  Home: 970-493-0837

- Dr. Mike Miller, DOW Veterinarian

  Office: 970-472-4348
  Cell: 970-566-4504
  Pager: 970-229-8792
  Home: 970-498-0998

- Dr. Lisa Wolfe, DOW Veterinarian

  Office: 970-472-4312
  Cell: 970-217-2328
  Pager: 970-206-7918
  Home: 970-498-0998